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Shorter Notes

The Gametophyte of *Lycopodiella prostrata*.—As part of an extended study on mycorrhizal and photosynthetic gametophytes of the Lycopodiaceae, spores of *Lycopodiella prostrata* (Harper) Cranfill, a species with an undescribed gametophyte, were cultured. The spores were obtained from plants collected in Cook County, Georgia and a voucher was deposited at VSU (*Carter #14616*). The conditions, techniques, and nutrient medium used were those of Whittier and Renzaglia (Amer. Fern J. 95:153–159. 2005). The system of classification followed in this report is that of Øllgaard (Opera Bot. 92:153–178. 1987).

There are five gametophyte types in *Lycopodium (s.l.)*. Four of the five are mycorrhizal with the following shapes – carrot-shaped, disk-shaped, uniaxial strap-shape, and branched cylindrical. The last type, which has been reported for *Lycopodiella*, is photosynthetic with a solid, more or less cylindrical base topped with photosynthetic lobes. This study was carried out to determine if the gametophyte of *L. prostrata* is this type.

Spore germination was slow. The earliest germination occurred two months after sowing spores in illuminated cultures, and at one year, 61 spores out of 10,000 (0.6%) had germinated. Spores cultured in the dark for one year did not germinate; however, spores from these dark cultures remained viable and 142 of them out of 10,000 (1.4%) germinated after moving them into the light for seven months.

Although spores of the mycorrhizal species of *Huperzia* and *Lycopodium* germinate slowly and at low percentages (Whittier, Amer. Fern J. 88:106–113. 1998), it is generally believed that *Lycopodiella* spores germinate rapidly and at high percentages (Whittier, Amer. Fern J. 88:106–113. 1998). This is not completely true because spores from some *Lycopodiella* species germinate slowly (Whittier, Amer. Fern J. 88:106–113. 1998).

Cell divisions in various planes formed a small mass of gametophyte tissue that remained partially contained by the spore coat. At about six weeks of growth, the young gametophyte escaped from the spore coat. At this time a small, dark green, ellipsoidal mass of cells formed – the young primary tubercle (Fig. 1A). Once the main body of the tubercle had a width of 150 μm or more, the first photosynthetic lobe developed at its apical end (Figs. 1B, 1C). Further enlargement of the tubercle resulted in a larger apical region where additional photosynthetic lobes formed. The lobes were erect, narrow, and strap-shaped with tapering distal ends.

The early mature gametophytes had a short, solid, more or less cylindrical base topped with numerous photosynthetic lobes. As the gametophytes aged, more lobes formed, and the previously formed lobes were displaced to the sides of the larger base. Gametophytes at this stage are illustrated in Figs. 1D and 1E.

The gametangia usually formed at the junction of the photosynthetic lobe and the gametophyte base. Both archegonia and antheridia developed on the

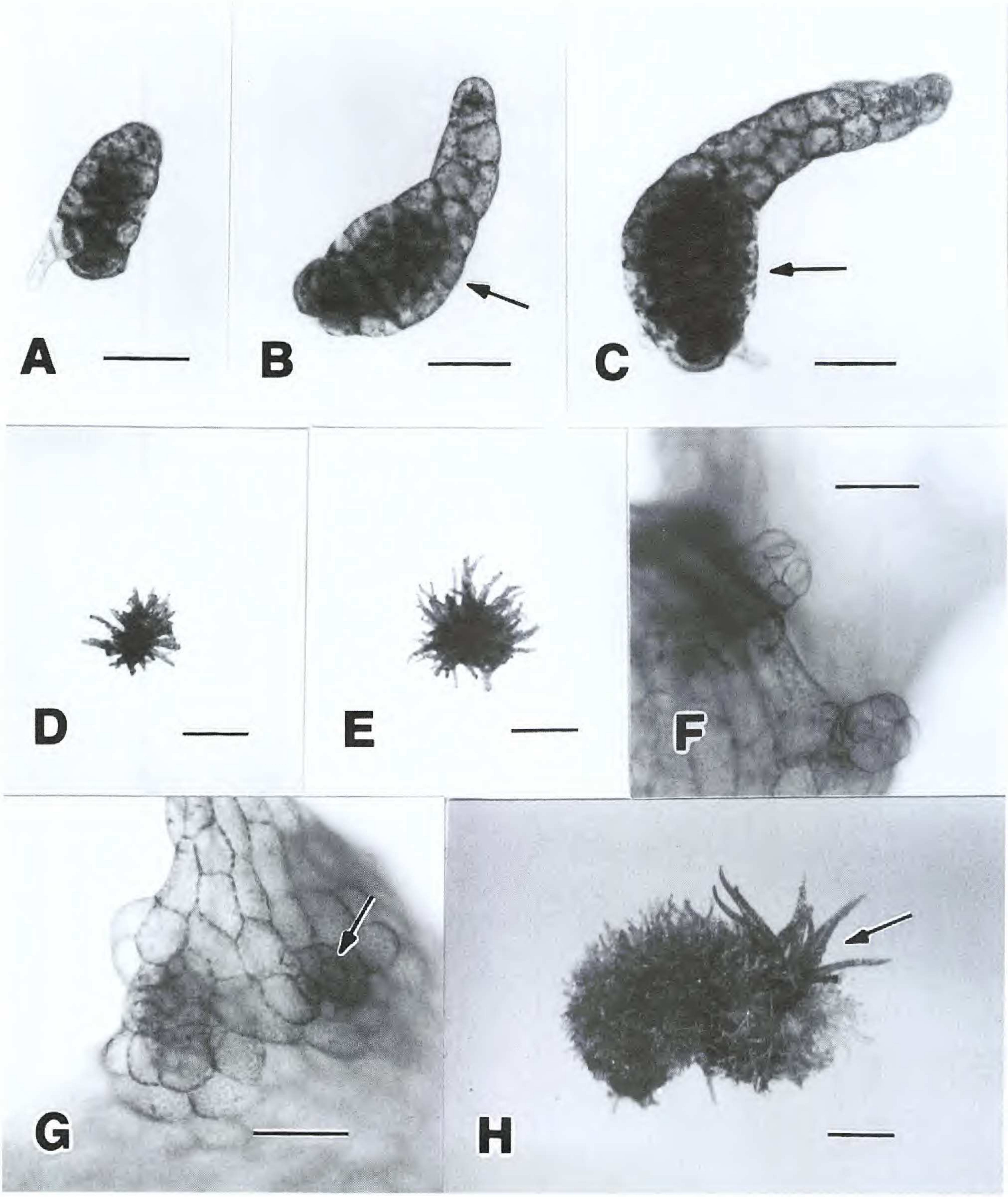

FIG. 1. Gametophytes of *Lycopodiella prostrata*. A. Primary tubercle. B–C. Primary tubercles (arrows) with young photosynthetic lobes (ca. 2 mo old). D. Oblique view of early mature gametophyte (ca. 5 mo old). E. Apical view of gametophyte (ca. 7 mo old). F. Two archegonia with short necks. G. Two antheridia – one with view of opercular cell (arrow). H. Large gametophyte (ca. 18 mo old) with young sporophyte (arrow). Bars = 100 µm for Figs. A–C & F–G, 1 mm for Figs. D–E, and 2 mm for Fig. H.

young mature gametophytes. The archegonia had short necks made up of two tiers of neck cells exposed above the gametophyte surface (Fig. 1F). The length of the archegonial neck was about 70 μm long. The length from the tip of the neck to base of egg was about 110 μm as determined with optical sections. Each antheridium had one opercular cell in the antheridial jacket at the gametophyte surface (Fig. 1G). Optical sections showed the gamete masses of the antheridia to be essentially spherical with diameters of about 70 μm.

The small, young gametophytes with both antheridia and archegonia continued to grow on the nutrient medium without undergoing sexual reproduction. With age these medium-sized gametophytes took on a pincushion shape (Fig. 1D, 1E). After a year or more in culture, large pincushion-shaped gametophytes formed. The solid basal portions of these gametophytes were obscured by the numerous photosynthetic lobes (Fig. 1H).

Mature gametophytes were capable of fertilization if water was added to the cultures. Fifty older gametophytes growing in separate cultures produced 24 sporophytes after flooding with water. The first microphylls, which were larger than the photosynthetic lobes, were evident two weeks after flooding. Within three months the young sporophytes became well established with numerous microphylls growing above the photosynthetic lobes (Fig. 1H).

The development of the primary tubercle is typical for *Lycopodiella* gametophytes and the ellipsoidal or oblong shape is known from other species (Whittier & Renzaglia, Amer. Fern J. 95:153–159. 2005). A growth from the top of the tubercle, the intermediate shaft, which was reported for *Lycopodiella* gametophytes growing on soil (Holloway, Trans. New Zealand Inst. 48:253–303. 1916; Bruce, Amer. J. Bot. 66:1156–1163. 1979), does not develop in *L. prostrata* under these conditions. It appears that the growth of *Lycopodiella* gametophytes in well-illuminated cultures prevents the development of the intermediate shaft (Whittier & Renzaglia, Amer. Fern J. 95:153–159. 2005).

Photosynthetic lobes develop from the top of the tubercle in *L. prostrata* as was observed with the gametophyte of *Lycopodiella lateralis* (R.Br.) B. Øllg. (Whittier & Renzaglia, Amer. Fern J. 95:153–159. 2005). The formation of the pincushion-shaped gametophyte with many green lobes arising from a solid base is typical for *Lycopodiella* (Wagner & Beitel, Flora North America 2:18–37. 1993). The young pincushion-shaped gametophytes with photosynthetic lobes arising from the apex and sides of the solid base appear to have a radial symmetry (Figs. 1D, 1E). The symmetry of the larger pincushion-shaped gametophytes (Fig. 1H) appears dorsiventral as was reported for *Lycopodiella carolinianum* by Bruce (Amer. J. Bot. 66:1156–1163. 1979). The long strap-shaped lobes have been described for *Lycopodiella* gametophytes previously (Whittier & Renzaglia, Amer. Fern J. 95:153–159. 2005).

Both gametangia form on these gametophytes at the base of the photosynthetic lobes. Descriptions of *Lycopodiella* archegonia indicate that they have short necks (Bruce, Amer. J. Bot. 63:919–924. 1976; Wagner & Beitel, Ann. Mo. Bot. Gard. 79:676–686. 1992). The antheridia are smaller than those reported for the terrestrial species of *Huperzia* (Whittier, Pintaud, & Braggins, Amer. Fern J. 95:22–29. 2005) and much smaller than those of *Lycopodium* (Bruce, Amer. J. Bot. 66:1138–1150. 1976; Whittier, Canad. J. Bot. 55:563–567. 1977). The gametangia of *Lycopodiella appressa* (F.Lloyd & L.Under.) Cranfill and *Lycopodiella cernua* (L.) Pichi-Serm. have essentially the same sizes as those of *L. prostrata*. The gametangia of *L. prostrata* are typical for *Lycopodiella*.

The development of the other types of gametophytes of the Lycopodiaceae is quite different from that found in *Lycopodiella*. The mature gametophyte of *Phylloglossum* is photosynthetic but it starts out as a subterranean, mycorrhizal gametophyte that is negatively gravitropic. After its exposure to light at the soil surface it becomes a green, bilaterally symmetrical, tuberous gametophyte lacking photosynthetic lobes (Whittier & Braggins, Amer. J. Bot. 87:920–924. 2000).

The remaining gametophytes of the Lycopodiaceae are subterranean, mycorrhizal, and nonphotosynthetic. Their development is initiated underground by the dark germination of their spores and requires a mycorrhizal association for continued growth. Early growth forms a solid, teardrop-shaped gametophyte that gives rise to the four other gametophyte shapes found in the Lycopodiaceae. Larger teardrop-shaped gametophytes develop ring meristems that form the radially symmetrical disk- and carrot-shaped gametophytes of *Lycopodium* (Whittier, Canad. J. Bot. 55:563–567. 1977; Whittier, Bot. Gaz. 142:519–524. 1981).

The uniaxial, dorsiventral, strap-shaped gametophyte of the terrestrial *Huperzia* species lacks a ring meristem. The meristem arises from a portion of the apical region of a larger teardrop-shaped gametophyte (Bruchmann, Flora 101:220–267. 1910). This meristem occurs in a subterminal groove overarched by young dorsal tissue on these strap-shaped gametophytes. With the epiphytic *Huperzia* species, the teardrop-shaped gametophyte enlarges and grows into the branched, cylindrical, mycorrhizal gametophyte (Whittier unpublished).

The gametophyte of *L. prostrata* has the typical structure and development of *Lycopodiella* gametophytes; thus it is different from the other gametophyte types of the Lycopodiaceae.—Dean P. Whittier, Department of Biological Sciences, Box 1634, Vanderbilt University, Nashville, TN 37235-1634, and Richard Carter, Department of Biology, Valdosta State University, Valdosta, GA 31698-0015.

Three New Flavonoid Glycosides, Kaempferol 3-*O*-(caffeoylrhamnoside), Apigenin 4′-*O*-(caffeoylglucoside) and 4′-*O*-(feruloylglucoside) from *Dryopteris villarii*.—Ten flavonol *O*-glycosides (based on kaempferol and quercetin), two flavanone *O*-glycosides (based on naringenin and eriodictyol) and three *C*-glycosylflavones (vitexin, vitexin 7-*O*-glucoside and orientin) have previously been identified by Hiraoka (Biochem. Syst. Ecol. 6: 171-175. 1978) in eighteen *Dryopteris* species whereas 3-desoxyanthocyanins have been found in red sori of *Dryopteris erythrosora* (Eat.) Kuntze by Harborne (Phytochemistry 5: 589–600. 1966). In addition kaempferol 7-*O*-(6″-succinyl-